CLINICO PATHOLOGICAL STUDY OF SMALL ROUND CELL TUMORS IN PEDIATRIC POPULATION

Dissertation submitted in partial fulfillment of the requirements for the degree of

M.D. (PATHOLOGY)
BRANCH - III

GOSCHEN INSTITUTE OF PATHOLOGY
MADRAS MEDICAL COLLEGE
CHENNAI.

THE TAMILNADU
DR.M.G.R.MEDICAL UNIVERSITY
CHENNAI

MARCH 2010

CERTIFICATE

This is to certify that this Dissertation entitled **"CLINICO PATHOLOGICAL STUDY OF SMALL ROUND CELL TUMORS IN PEDIATRIC POPULATION"** is the bonafide original work of Dr.N.PRIYATHERSINI, in partial fulfillment of the requirement for M.D., (Branch III) in pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in March 2010.

Prof.Dr.J.MOHANA SUNDARAM, M.D., D.N.B. Ph.D.,
DEAN
Madras Medical College and
Government General Hospital
Chennai – 600003

Prof.Dr.A.SUNDARAM, M.D.,
DIRECTOR,
Institute of Pathology
and Electron Microscopy
Madras Medical College
Chennai – 600003

DECLARATION

I Dr.N.Priyathersini, solemnly declare that the dissertation titled **"CLINICO PATHOLOGICAL STUDY OF SMALL ROUND CELL TUMORS IN PEDIATRIC POPULATION"** is the bonafide work done by me at Institute of Child Health, Madras Medical College under the expert guidance and supervison of Dr. A.Sundaram M.D, Professor and Director of Institute of Pathology and Electron Microscopy, Madras Medical College. The dissertation is submitted to the Tamilnadu, Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in pathology.

Place : Chennai
Date :

Dr. N.PRIYATHERSINI

ACKNOWLEDGEMENT

I express my sincere thanks to **Prof.Dr.J.MOHANA SUNDARAM, M.D., D.N.B., Ph.D.,** Dean, Madras Medical College and Government General Hospital, for permitting me to utilize the facilities of the Institution.

I take this opportunity to express my heart felt gratitude to **Dr.A.SUNDARAM, M.D.,** Professor and Director of Institute of Pathology and Electron Microscopy, Madras Medical College, Chennai for his keen interest, constant encouragement, guidance and valuable suggestions throughout the study.

I am extremely thankful to **Dr.V.RAJALAKSHMI, M.D., D.C.P.,** Professor and Head of the department of Pathology, Kilpauk Medical College, Chennai, former Professor of Pathology, Institute of Child Health who has expertly guided me in this project for her unstinted encouragement and valuable suggestions during the study.

My sincere thanks to **Dr.P.KARKUZHALI, M.D.,** Professor of Pathology, Institute of Pathology and Electron Microscopy, Madras Medical College for her expert advice, encouragement and suggestions during the study.

I am truly thankful to **Dr.GEETHA DEVADAS, M.D., D.C.P.,** Professor of Pathology, Institute of Pathology and Electron Microscopy, Madras Medical College for her constant cheer and support throughout the study.

I thank **Dr.S.PAPPATHI, M.D., D.C.H.,** Professor of Pathology, Institute of Pathology and Electron Microscopy, Madras Medical College for all her help and encouragement during the study.

I take the opportunity to express my thanks to **Dr.SUDHA VENKATESH, M.D.,** Professor of Pathology, Institute of Pathology and Electron Microscopy, Madras Medical College for her opinions and encouragement throughout the study.

I thank **Dr.T.B.UMADEVI, M.D.,** Professor of Pathology,Institute of Child Health, Madras Medical College for her support during the course of the study.

I express my heartfelt thanks to **Dr.SHANTHA RAVISANKAR, M.D.,** Professor of Neuro- Pathology,Institute of Neurology, Madras Medical College for her constant abets, direction and valuable advice during the study.

My thanks to **Dr.M.P.KANCHANA, M.D.,** Professor of Pathology, Institute of Obstetrics & Gynecology, Madras Medical College for all her encouragement and opinions about the study.

I convey my thanks to **Dr.K.RAMA, M.D.,** Professor of Pathology,Govt. Kasturba Gandhi Hospital, Madras Medical College for her suggestions and support during the period of study.

I put across my thankfulness to **Dr.T.CHITRA, M.D.,** Professor of Pathology, Regional Institute of Ophthalmology, Madras Medical College, for her abets and aid during the study period.

I express my sincere thanks to all my Assistant Professors for their help and suggestions during the study.

I am thankful to all my Colleagues, Friends, Technicians and Staff of the Institute of Pathology and Electron Microscopy, Madras Medical College, Chennai for all their help and support they extended for the successful completion of this dissertation.

ABBREVIATIONS

SRCTs	:	Small Round Cell Tumors
NHL	:	Non Hodgkins Lymphoma
ES	:	Ewings Sarcoma
PNET	:	Peripheral Primitive Neuroectodermal Tumors
NB	:	Neuroblastoma
RMS	:	Rhabdomyosarcoma
DSRCT	:	Desmoplastic Small Round Cell Tumors
BWT	:	Blastemal Wilms Tumor
IHC	:	Immunohistochemistry
RT-PCR	:	Reverse Transcriptase Polymerase Chain Reaction

CONTENTS

INTRODUCTION

Malignancies of childhood include a well-defined spectrum of hematolymphoid neoplasms, organ specific (adrenal, kidney, liver) neoplasms, soft tissues, bone and nervous system (central and peripheral) neoplasms with variable biology. Small round cell tumors (SRCT) are a subset of childhood malignancies that comprise a group of diverse, diagnostically challenging primitive or undifferentiated neoplasms. These tumors have a similar histological appearance that is small round blue cells. They share many morphological similarities, however their unique biological, immunohistochemical, ultrastructural and genetic characteristics have provided substantial insights into the pathology of these diverse neoplasms. Traditionally clinical features and light microscopy with aid of histochemistry and ultrastructural evaluation, establish a diagnosis. Additionally, immunohistology, cytogenitics and molecular studies have become important in diagnosis and in defining phenotype / genotype, patient treatment modalities and prognosis in specific cases.

AIMS AND OBJECTIVES

1. To study the incidence of small round cell tumors in pediatric population.
2. To study the clinical and histopathological features of small round cell tumors.
3. To confirm the histopathological diagnosis with immunohisto-chemistry wherever it is possible.

REVIEW OF LITERATURE

Definition of SRCTS of childhood

Denny in his commentary defines the characteristics of small round cell tumors (SRCT)[1].

SRCTs can be defined in terms of clinical and pathological features i.e. the routine histological, immunohistochemical and ultrastructural features and by genetic markers. Although microarray analyses can discriminate between types of SRCTs, a competent pathologist can do it with equal accuracy by using routine histological and ancillary morphologic techniques.

SRCTs constituting about 20% of the solid tumors in children include the following[2].

Small round cell tumors –Table 1

BASIC CONVENTIONAL SPECTRUM

1. Neuroblastoma (NB)
2. Ewings Sarcoma/Peripheral primitive Neuroectodermal Tumors (ES/PNET)
3. Rhabdomyosarcoma (RMS)
4. Non Hodgkins lymphoma (NHL)

EXTENDED SPECTURUM

1. Desmoplastic SRCT (DSRCT)
2. Small cell undifferentiated Osteosarcoma (UOS)
3. Small cell Hepatoblastoma (HBI)
4. Blastemal Wilms Tumor (BWT)
5. Small cell variant of malignant peripheral Nerve sheath tumor (MPNST)
6. Small cell variant of synovial sarcoma (SS)
7. Unclassifiable SRCT

Precise diagnosis of the tumor type is necessary for selection of appropriate treatment protocol. The clinical features, primary site, metastatic pattern and routine pathologic features provide clues to the diagnosis whereas other features which include immunohistochemical, ultrastuctural and genetic features provide definitive criteria for diagnosis. The main emphasis is on the various parameters for the basic conventional spectrum in children (Table 1). The features that prove helpful in the diagnosis of the entities in the extended spectrum of SRCTs in children with be described briefly.

EPIDEMIOLOGY OF CHILDHOOD CANCERS

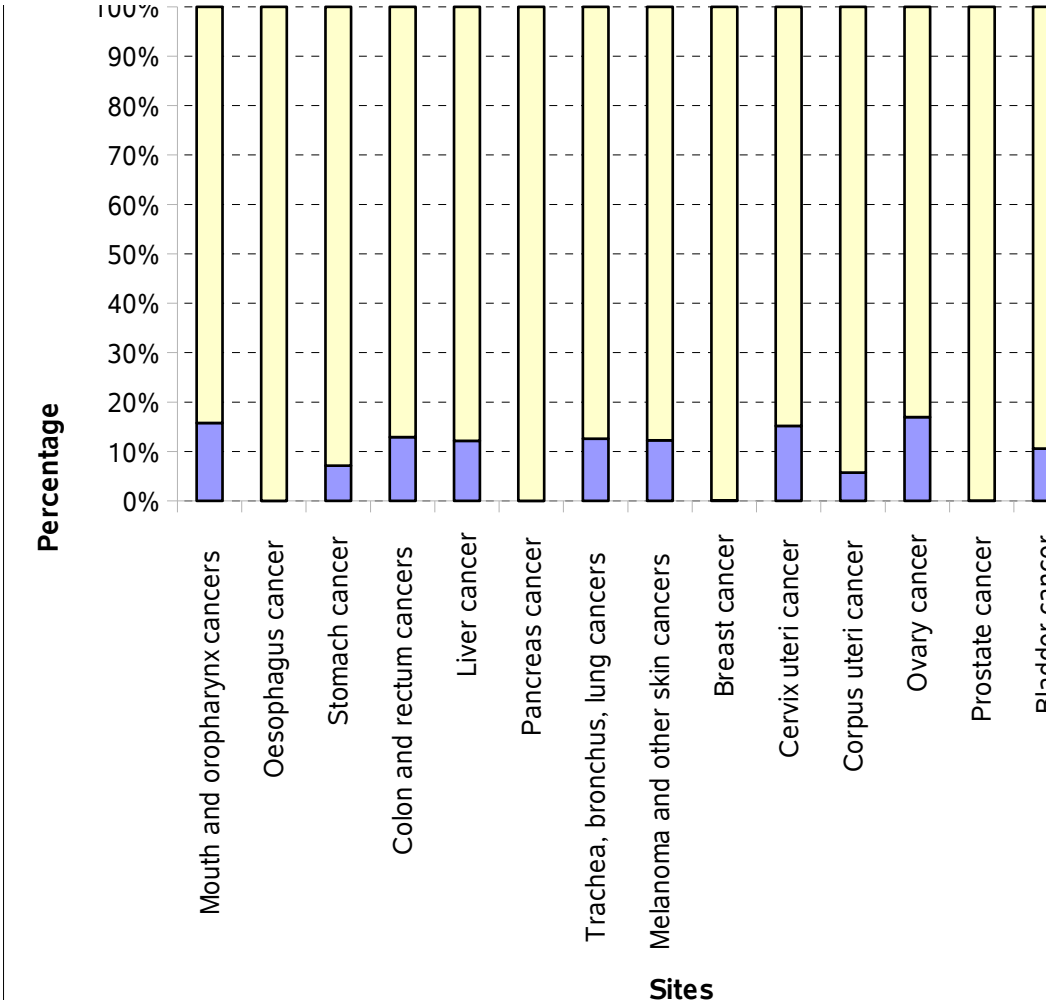
The incidence of childhood cancer in most populations in the world ranges from 75 to 150 per million children per year.[3,4] However, the reported age of the standardized incidence rate for India ranges from 38 to 124 per million children per year.The highest incidence is reported from Chennai and the lowest from rural Ahmedabad. The estimated DALYs the world paediatric population is given in the table-2. The incidence of tumours is given in the Figure-1.

TABLE - 2
Estimated DALYs ('000), by cause and WHO Member State, persons aged 0-14 years, 2004

	India	World
Malignant neoplasms	**595**	**3,202**
Mouth and oropharynx cancers	9	47
Oesophagus cancer	0	6
Stomach cancer	1	15
Colon and rectum cancers	2	13
Liver cancer	12	90
Pancreas cancer	0	3
Trachea, bronchus, lung cancers	3	21
Melanoma and other skin cancers	1	9
Breast cancer	0	4
Cervix uteri cancer	0	2
Corpus uteri cancer	0	4
Ovary cancer	7	32
Prostate cancer	0	3
Bladder cancer	1	6
Lymphomas, multiple myeloma	85	615
Leukaemia	271	1,226

Other neoplasms	**56**	**317**

Figure - 1


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage
Mouth and oropharynx cancers
Oesophagus cancer
Stomach cancer
Colon and rectum cancers
Liver cancer
Pancreas cancer
Trachea, bronchus, lung cancers
Melanoma and other skin cancers
Breast cancer
Cervix uteri cancer
Corpus uteri cancer
Ovary cancer
Prostate cancer
Sites

VARIATION BY SEX

Overall cancer in childhood is more common among males than females and the male to female ratio in the most resource-rich countries is around 1.2:1.[5,6] However, some cancers like retinoblastoma, Wilms' tumor, osteosarcoma, and germ cell tumor actually show a slight female preponderance.

VARIATION BY CANCER TYPE

Leukemia is the most common childhood cancer in India with relative proportion varying between 25 and 40%. Sixty to 85% of all leukemias reported are acute lymphoblastic leukemia (ALL).
In the developed world, CNS tumors are the second most common childhood cancer (22-25%) and lymphomas a distant third (10%).[5,6] In contrast, in India lymphomas often exceed CNS tumors, particularly in males. Not only is the proportion of lymphomas higher in India, but HD exceeds non-Hodgkin's lymphoma (NHL), a pattern opposite to that seen in the developed world. This specifically seems to be a result of the high incidence of HD in male children in India (incidence rate of 8.2-19.6 per million children, per year, in Bangalore, Chennai, Delhi, and Mumbai PBCRs compared to 5.7 in USA and 6.4 in Britain).[5,6] In contrast, the

incidence of HD in females and of NHL in both sexes is not very different from the incidence in the developed world. Besides differences in incidence, the pathobiology of these cancers is also different. Among NHL, the proportion of T-cell lymphoblastic lymphoma and diffuse large B-cell lymphoma is much higher and the proportion of mature B-cell (Burkitt's and Burkitt-like) lymphoma much lower in India than that seen in the developed world.[7] Similar to T-cell ALL, the higher proportion of T-cell NHL may be linked to lower socioeconomic status. Mixed cellularity is the most common Hodgkin's disease subtype and is responsible for the incidence peak at a younger age, as seen in India, compared to the peak seen at ages 16 to 30 years in the developed world, where nodular sclerosis is most common.[4] The high proportion of mixed cellularity in India is thought to be related to early childhood Ebstein Barr virus exposure.[8]

Neuroblastoma, which is the second most common solid tumor in childhood after CNS tumors,, is much less frequently reported in India. Retinoblastoma has an incidence rate of three to five per million children, per year, and accounts for 2.5 to 4% of all childhood cancers in most developed countries. Barshi, Chennai, and Delhi report a 2-3 fold higher incidence of tumors of the eye (majority of which will be retinoblastoma

in children <15 years of age), a finding that has also been previously reported.[9,10] Variation is also seen in the incidence of tumors of the kidney (majority of which are Wilms' tumor in children <15 years age), with the incidence in Bangalore and Mumbai being half of that seen in Delhi, Chennai, and other developed countries.[11] Incidence and relative proportion of specific types of cancer in childhood in Chennai is given in the Table – 3.

TABLE - 3

Number (#) and relative proportion (%) of specific types of cancer in childhood Age (0-14 years) (2001-03) – Location Chennai [12]

Specific Types of Cancer in Childhood	#	%
I LEUKAEMIAS	280	42.2
(a) Lymphoid Leukaemia	193	29.1
(b) Acute non-lymphocytic leukaemia	57	8.6
(c) Chronic myeloid leukaemia	11	1.7
(d) Other specified leukaemias	1	0.2
(e) Unsp. Leukaemias	18	2.7
II LYMPHOMAS & RETICULOENDOTHELIAL NEOP.	109	16.4
(a) Hodgkin's disease	58	8.7
(b) Non-Hodgkin lymphoma	**43**	**6.5**
(c) Burkitt's lymphoma	**8**	**1.2**
(d) Misc lymphoreticular neop.	0	0.0
(e) Unsp. Lymphomas	0	0.0
III C.N.S. & MISC. INTRACRANIAL & INTRASPINAL NEOP.	30	4.5
(a) Ependymoma	3	0.5
(b) Astrocytoma	11	1.7
(c) Primitive neuroectodermal tumors	6	0.9
(d) Other gliomas	7	1.1
(e) Other specified intracranial and intraspinal neop.	0	0.0

(f) Unsp. intracranial and intraspinal neop.	3	0.5
IV SYMPATHETIC NERVOUS SYSTEM TUMOURS	16	2.4
(a) Neuroblastoma and Ganglioneuroblastoma	**15**	**2.3**
(b) Other SNS tumors	1	0.2
V RETINOBLASTOMA	43	6.5
VI RENAL TUMOURS	23	3.5
(a) Wilms's tumor,rhabdoid and clear cell sarcoma	22	3.3
(b) Renal carcinoma	1	0.2
(c) Unsp. malignant renal tumors	0	0.0
VII HEPATIC TUMOURS	4	0.6
(a) Hepatoblastoma	3	0.5
(b) Hepatic carcinoma	1	0.2
(c) Unsp. malignant hepatic tumours	0	0.0
VIII MALIGNANT BONE TUMOURS	65	9.8
(a) Osteosarcoma	40	6.0
(b) Chondrosarcoma	0	0.0
(c) Ewing's sarcoma	**22**	**3.3**
(d) Other specified malignant bone tumours	1	0.2
(e) Unsp. malignant bone tumours	2	0.3
IX SOFT-TISSUE(S-T) SARCOMAS(S)	40	6.0
(a) Rhabdomyosarcoma and embryonal sarcoma	**22**	**3.3**
(b) Fibros.neurofibros. and other fibromatous neop.	1	0.2
(c) Kaposi's sarcoma	0	0.0
(d) Other specified soft tissue sarcomas	4	0.6
(e) Unsp. soft tissue sarcomas	13	2.0
X GERM-CELL TROPHOBLASTIC & OTH. GONADAL NEOP.	18	2.7
(a) Intracranial and intraspinal gc tumours	0	0.0
(b) Other and unsp. non-gonadal gc tumours	0	0.0
(c) Gonadal gc tumours	0	0.0
(d) Gonadal carcinomas	4	0.6
(e) Other and unsp. gonadal tumours	14	2.1
XI CARCINOMA & OTH MALIGNANT EPITHELIAL NEOP.	24	3.6
(b) Adrenocortical carcinoma	4	0.6
(a) Thyroid carcinoma	1	0.2
(c) Nasopharyngeal carcinoma	10	1.5
(d) Malignant melanoma	0	0.0
(e) Skin carcinoma	6	0.9
(f) Other and unsp. Carcinomas	3	0.5
XII OTHER & UNSP. MALIGNANT	12	1.8

NEOPLASMS		
(a) Other specified malignant tumours	0	0.0
(b) Other unsp. malignant tumours	12	1.8
XIII. OTHERS (Not Classified)	0	0.0
All Types	664	100.0

INCIDENCE OF SRCT

SRCTs constitute around 20% of all the solid tumors in the children[2].

Lymphomas are the third common malignancies of children after acute leukemias and brain tumors[3]. Neuroblastoma is the most common extra cranial solid tumor in children. It occurs at a rate of about 1 per 10000 live births[14]. It accounts for 10-20% of all malignant tumors.

Ewing's sarcoma/PNET constitute around 6-10% of primary malignant bone tumors. Extra skeletal Ewings constitute the second common pediatric soft tissue sarcoma[6]. Rhabdomyosarcoma is the most common pediatric soft tissue sarcoma comprising 80-90% of reported cases[17].

Age adjusted incidences of selected cancers per 100000 individuals aged 0-19 years are as follows:[13]

All Sites – 15.9

Leukemias – 3.8

Brain and other nervous tissues – 2.8

Hodgkin disease – 1.3

Non-Hodgkin lymphoma – 1.1

Bone and joint – 1

Soft tissue – 1

Kidney and renal pelvis – 0.7

AGE AND SEX

Non-Hodgkin lymphoma[18] is less common than that of Hodgkins lymphoma in children. However NHL and acute leukemias are best considered in terms of a spectrum ranging from clinically localised disease to overt leukemia. Neuroblastomas[15] are generally diagnosed in early childhood. 50% are below 2 years and 90% of cases below 5 years. There is a slight male predilection.

Ewings sarcoma/PNET[15] have average age presentation of 10-15 years. 80% are less than 20 years. Boys are affected more frequently than girls at a ratio of 1.3:1. Rhahdomyosarcoma[19] in children occurs principally before the age of 10 years with a peak before the age of 4 years and shows moderate male predominance.

PRIMARY SITE AND METASTATIC PATTERNS

The most characteristic sites for the SRCTs in the basic conventional spectrum are as follows.[2]

a) Adrenal - Neuroblastoma

b) Bone - ES/PNET

c) Lymph nodes - Non Hodgkins Lymphoma (NHL)

d) Soft tissues - RMS

However exceptions occur like Neuroblastoma of kidney, NHL of bone. In rare cases of Neuroblastoma and RMS the primary site may be occult.

The characteristic metastatic patterns are as follows.

a) Bone marrow, lymph node liver, orbit and bone – Neuroblastoma

b) Lung and lymph node – ES/PNET & RMS

c) Generalized disease – NHL

However exceptions do occur. Lung and Bone marrow may be the site of metastases of Neuroblastoma and intracranial SRCT such as central PNET including medulloblastoma may metastasize extra cranially to lungs, liver and kidneys. Knowledge of typical metastatic patterns of various SRCTs can provide a clue. However, the primary tumors at these sites and unusual metastatic patterns will also have to be considered. (e.g. Hepatoblastoma in liver, NHL or metastatic undiffentiated nasopharyngeal carcinoma in cervical lymphnodes, small cell melanoma

or small cell sweat gland carcinoma or metastatic NB in the biopsy of a skin nodule.**)**

SIGNS AND SYMPTOMS

In general patients with NHL[18] appear mildly to moderately ill with low-grade fever, pallor, respiratory distress, pain and discomfort. Usually present with generalized lymphadenopathy or with abdominal mass. Uncommonly may present with nasopharyngeal mass, or parotid enlargement, nephromegaly or testicular enlargement.

Neuroblastoma[18] mostly presents as an abdominal mass in which radiologic imaging studies demonstrate a suprarenal or retroperitoneal mass with or without calcifications. Dehner et al enumerates some of the uncommon and unusual clinical manifestations of Neuroblastoma:

1. Congenital Neuroblastoma
2. Cutaneous metastasis
3. Fetal hydrops with placental metastases
4. Neuroblastoma " Leukemia"
5. Opsoclonus – myoclonus with: dancing eyes"

6. Systemic Hypertension
7. Intractable watery diarrhea (VIP secretion)
8. Heterochromia of iris (Horner syndrome)
9. Cushing syndrome
10. Familial Neuroblastoma
11. Late recurrence and death

Ewings sarcoma[7] typically presents as a painful enlarging mass with features mimicking infection like fever, elevated erythrocyte sedimentation rate, anemia and leukocytosis. Plain X-ray study reveals a destructive lytic lesion, eroding the cortex producing “saucerisation” of the bone and the displaced periosteum deposits layers of reactive bone in an onion- skin like fashion.

Rhabomyosarcomas[8] usually manifests as an expanding mass. Typical presentations by the location of non-metastatic disease are as follows:

Orbit	-	Proptosis or dysconjugate gaze
Paratesticular	-	Painless scrotal mass

Prostate	-	Bladder or bowel difficulties
Uterus, Cervix, Vagina	-	Menorrohagia or metrorrhagea
Bladder	-	Protruding polypoid mass (Botryoid meaning a grapelike cluster)
Extremity	-	Painless mass
Parameningeal (ear, Nasal cavity, paranasal Sinuses, infratemporal fossa, Pterygopalatine fossa)	-	Upper respiratory symptoms or pain

GROSS FEATURES

Neuroblastomas are lobulated masses averaging 6-8cm in diameter[6]. They often have a delicate membranous capsules that are easily ruptured to yield the soft, fleshy, gray partially hemorrhagic tumor.

Ewings sarcoma/PNET[7] arises in the medullary cavity usually transgresses the cortex and periosteum producing a soft tissue mass. The tumor is tan white or fish flesh like appearance and frequently contains areas of hemorrhage and necrosis.

Rhabdomyosarcomas[6] are generally fleshy non descript pale gray-yellow masses with areas of necrosis and hemorrhage. Of particular note is the gross appearance of the botryoid variant of rhabdomyosarcoma because of its resemblance to cluster of grapes. The tumor arises exclusively along mucosa lined surfaces such as the bladder or vagina.

Non-Hodgkin lymphomas[8] generally present as generalized lymphadenopathy with hepatosplenomegaly. The nodes are enlarged solid homogenous grey mass with cut surface resembling cut potato appearance**.**

MICROSCOPIC FEATURES

At the time of incisional biopsy a frozen section diagnosis of small round cell tumor, identification of precise type deferred till permanent section, is justified in most cases of SRCT.

In routine histologic sections, clues are provided by patterns and nuclear and cytoplasmic features[2]. (Table 4)

PATTERNS OF TUMOR CELLS

1. Diffuse – NHL, ES/PNET

2. Well-developed nesting (lobular) pattern with fibro vascular septa (NB)

3. Filigree pattern or focal nesting pattern (ES/PNET)

4. Pseudofiligree pattern due to crust artifact (any SRCT)

5. Cambium layer in a polyploidy tumor (Botryoides sarcoma)

6. Myxoid pattern (Embryonal RMS)

7. Osteoid formation (Small cell Osteosarcoma)

8. Rare tubular structure (Blastemal WT, small cell synovial sarcoma)

9. Well – developed rosettes (NB)

10. Ill – defined rosettes (FS/PNET)

11. Eosinophilic fibrillary background of neuropil(NB)

12. Alveolar pattern: Alveolar RMS (alveolar pattern is absent or only focally present in the solid variant of alveolar RMS)

13. Starry sky pattern (NHL, rarely NB)

14. Non – cohesive pattern (NHL)

15. Desmoplasia (Desmoplastic SRCT)

16. Mucoid Background (Small cell Hepatoblastoma)

CYTOLOGICAL FEATURES

TABLE - 4

Feature	**SRCT in which the feature is characteristically seen.**
Large round cells	ES, large cell NHL, alveolar RMS
Spindle shaped or fusiform cells	Embroynal RMS, rare cases of NB, ES/PNET, Blastemal WT
Convoluted Nuclei	Lymphoblastic NHL
Peripheral prominent nucleoli	Large Cell NHL
Central Prominent nucleoli	Immunoblastic NHL
Lymphoglandular bodies	NHL
Mitotic Karryorrectic cells	NB, NHL
Thin rim of basophilic cytoplasm	Burkitt's NHL
Thin rim of clear cytoplasm due to prominent glycogen content	ES / PNET
Vacuoles in cytoplasm	Burkitt's NHL

There are small round cell variants of tumors which are usually composed of spindle – shaped or fusiform cells (synovial sarcoma and MPNST). There is also a well- characterized spindle cell type of embryonal RMS.

The strongest clue to the diagnosis of SRCT is provided by the differentiating (or rarely differentiated) cells, which may be sparse. These include:

a) **Rhahdomyoblasts and strap cells:** Showing dense eosinophilic cytoplasm with cross striations.

b) **Ganglion cells or cells intermediate between undifferentiated neuroblasts and ganglion cells:** Cells with vesicular nucleus, small neucleolus and discernible cytoplasm) in NB.

HISTOCHEMISTRY

Certain histochemical features of the tumors may help to define cytological features.

PAS with and without diastase demonstrates glycogen in ES/PNET

Masson's trichrome PTAH demonstrates cross-striations of rhabdomyoblasts.

The current standard of practice of surgical pathology requires that the precise nature of tumor cells be confirmed by more definitive objective, reproducible, immunohistochemical ultrastructural and/or molecular biologic (genetic) markers.

IMMUNOCYTOCHEMISTRY IN DIAGNOSIS OF SMALL ROUND CELL TUMORS OF CHILDHOOD

Based upon clinical information, tumor site, touch preparations or possibly frozen section examination and SRCT antibody panel may be ordered at the time of gross examination, so that the tissue sections and immunocytochemical studies are initiated as soon as possible following tissue processing. Evaluation of the routinely stained permanent sections, allow for altering the antibody panel to confirm a specific tumor type or to eliminate other diagnostic categories not considered previous.

The immunocytochemical profiles of conventional and extended spectrums of SRCTs and aberrant immunoreactivily of SRCTs are presented in tabular form.[20-36] (Table – 5 & 6)

INITIAL ANTIBODY PANEL

Myogenin or Desmin

NB84 or Neuron specific Enolase

Leucocyte Common Antigen

CD99

Vimentin

Alpha fetoprotein

TABLE - 5

ANTIBODIES AND IMMUNOREACTIVITY OF SMALL ROUND CELL TUMORS CHILDHOOD

Antibody	**Tumors Typically Immunoreact with Antibody**	**Tumors may Immunoreact with Antibody**
Leukocyte common Antigen	Lymphoid Leukemia, Lymphoma	Mesenchymal Chondrosarcoma
S-100 Protein	Neuroblastoma, MPNST Ewings sarcoma /PNET	DSRCT, Hepatoblastoma, Wilms Tumor, Synovial sarcoma, Rhabdomyosarcoma Myofibroma, Mesenchymal chondrosarcoma, small cell Osteosarcoma
NB84	Neuroblastoma	DSRCTs, ES
Neuron specific Enolase	Neuroblastoma, DSRCT	Rhahdomyosarcoma, Ewings sarcoma
Myogenin	Rhabdomyosarcoma	DSRCTs, Wilms Tumor
Desmin	Rhabdomyosarcoma, DSRCT	Wilms Tumor / MPNST, Rhabdoid Tumor
Muscle specific Actin	Rhabdomyosarcoma, Myofibroma	DSRCT, Wilms tumor, Rhabdoid tumor, MPNST
CD99	Ewings Sarcoma /PNET	Leukemia, Lymphoma, RMS, DSRCTs, Synovial sarcoma, Mesenchymal chondrosarcoma
Pancytokeratin	Synovial sarcoma, Hepato blastoma, Carcinoma, Rhabdoid Tumor, Germ cell tumors, DSRCT	Ewings sarcoma, RMS, Leukemia, lymphoma
Alpha – Fetoprotein	Hepatoblastoma, Endodermal sinus tumor (Yolk sac Tumor)	
Myeloperoxidase	Myeloid Leukemia	

Antibody panels for defining origin of undifferentiated SRCT[20-36].

Myogenic:	Desmin
	Myogenin
	Muscle Specific Actin
Neural:	NB84
	Neuron specific endase
	S100
Lymphoid:	Leukocyte Common Antigen
	Myeloperoxidase protein
Germ Cell:	Alpha feloprotein
	Pancytokeratin
Neural Crest:	S100 Protein
	HMB-45
	CD99
Mesenchymal:	Vimentin
	Smooth muscle Actin

TABLE - 6
CONVENTIONAL SPECTRUM OF SRCTs: IHC IMMUNOREACTIVITY

	Typical	Possible	Aberrant
Neuroblastoma	NB84 Neuron specific enolase Chromogranin Synaptophysin S-100 Protein Leu 7(CD57) Glial Fibrillary Protein Neural cell Adhesion Molecule Ganglioside G-D2	Neurofilament Triple protein Microtubule Associated Protein Dopamine Peripherin Vasoactive Intestinal Protein Protein Genen product 9.5 Myelin Basic Proein Trk - A	Vimentin
ES/PNET	CD99 Beta 2 microglobulin Vimentin Acetylcholine	S-100 Protein Neuron Specific Enolase Neurofilament Triple Protein Synaptophysin Leu7(CD57) Chromogranin Glial Fibrillary Acid Protein Neurofilament Triple protein	Pancytokeratin Desmin MSA EMA NB84 TrkAu
Rhabdomyosarcoma	Myogenin Desmin MSA MyoD1 Vimentine	SMA Dystrophic Creatine Kinase M Subunit Calsequestrin Myf-3 and Myf-4 Myoglobin	S100 Protein Pancytokeratin CD19/CD20/CD99 EMA NSE Leu7(CD57) CD68 Trk A
Non Hodgkins Lymphoma/Lymphoid Leukemia	Leukocyte common antigen CD3/CD4/CD8/CD45 RO(T-Cell) CD19/CD20/CD79a (B-Cell)	ALK-NPM(ALK-1-P80) CD-30(Ki-1,BerH2). Vimentin	Cytokeratin EMA

ANTIBODIES ASSOCIATED WITH CHIMERIC AND TUMOR SUPPRESSOR PROTEINS

Several antibodies capable of detecting cytogenetic translocation and tumor suppressor proteins are available for formalin-fixed, paraffin embedded tumor tissues. Recently antibodies (ALK-1 and P80) to the chimeric protein produced by the translocation [t(2:5), AI-NPM] associated anaplastic large cell lymphoma may provide a means for expected diagnosis via immunocytochemistry [2]. The mutated tumor suppressor WT-1 has been identified in 40% of Wilm's tumors and a large proportion of DSRCTs. P53 protein overexpression in several SRCTs, including rhabdomyosarcoma and Wilms tumor has been associated with unfavourable histology, recurrences, metastatic disease and decreased survival. Over expression of the retinoblastoma gene protein, PRB, may be seen in SRCTs and may have diagnostic value in certain tumors such as small cell (Undifferentiated) osteosarcoma.

PROLIFERATION MARKERS

Many proliferation markers associated with the cell cycle have unfavorable prognostic significance[20,31,34,36,37]. The overexpression of MIB1(Ki-67), PCNA, bcl-2, p15, p16 and cyclin dependent kinases are

associated with higher grade and stage of tumor, as well as unfavorable outcome. In the future, semi quantitative and more rigorous quantitative analysis of tumor suppressor gene products and cell cycle proliferation markers may become a standard of care.

ELECTRON MICROSCOPY IN SRCTS

Even when a pediatric round cell tumor appears completely undifferentiated upon light microscopy, ultrastructural diagnosis is normally quite simple and straightforward. The characteristic ultra structural features of SRCTs include the following[38,39,40,41] (Table-7)

ULTRA STRUCTURAL DIAGNOSTIC FEATURES

TABLE - 7

	Intercellular junctions	**External Lamina**	**Glycogen**	**Neurosecretory granules**	**Myofilament**
NB	+	-	-	+	-
RMS	-	+	+	-	+
ES/PNET	+	-	+	+* -	-
NHL	-	-	-	-	-

*A few neurosecretory granules may be present in PNET.

IMMUNOHISTOCHEMISTRY VS ELECTRON MICROSCOPY

Electon microscopy like standard light microscopy provides a direct morphological approach and is therefore the most powerful and least treacherous of the many ancillary diagnostic techniques available[33]. While other ancillary techniques [eg. IHC,FISH,PCR] are restricted in application to hypothesis testing electron microscopy can provide a correct answer even when the wrong question or no specific question is being asked. This having been said it must be stressed that each of these special techniques has its relative strength and weakness in particular situations[44,45].These should not be considered as competitive techniques but rather as complementary tools, which are best employed using a fully intergrated approach.

MOLECULAR PATHOLOGY OF SRCTS

Molecular markers have been increasingly used as a diagnostic tools as well as indicators for prognosis. Molecular markers that are used to diagnose a SRCT is given below[46-54]. (Table – 8)

TABLE - 8

MOLECULAR MARKERS USED IN DIAGNOSIS & PROGNOSIS OF SRCTS

Tumor	Marker	Locus/Trans location	Comments/Techniques
NB	MYCN	2p24	Amplification (> 10 copies), poor prognosis, usually associated with high stage disease; PCR or southern blot.
NB	Del 1p	1p3l-1p32	Associated with high stage disease; smaller deletion associated with more favorable outcome; PCR
ES/PNET	EWS/FLI-1	(11,22)(q24; q12)	Found in upto 90% cases; Types I and II fusion products most common; Type I more favorable; RT-PCR
ES/PNET	EWS/ERG	(21,22) (q22:q21)	Found in up to 5% of cases; RT-PCR
NHL, B-Cell type	IgH	14q32	Gene rearrangements identified in > 90% of cases; PCR or southern blot
NHL, T-Cell type	TCR or	7q34 or 7p15	Rearrangements identified in majority of cases; PCR or Southern blot
Wilms Tumor	WT1	11p13	Mutations occur in both sporadic and hereditary Wilms Tumors; PCR
DSRCT	EWS/WT-1	(11,22) (q113; q12)	Only found in DSRCT; RT-PCR
Alveolar Rhabdomyosarcoma	PAX3/FKHR	(2; 13)(Q35; q14)	Found in upto 70% of cases; 20% cases have t(1:13)(p36;q14), PAX7/FKHR associated with better prognosis; RT-PCR
Synovial sarcoma	SYT/SSX	(X:18) (P11:Q11)	80% of cases; SYT/SSX1 in biphasic sarcomas. SYT/SSX2 in monphastic type associated with better prognosis: RT-PCR

In addition to the molecular markers outlined in this table for broad groups of B and T cell NHL, numerous abnormal translocations with fusion of genes and resulting fusion transcripts have been demonstrated in specific subtypes of B and T cell NHL.

UNCLASSIFIABLE TUMORS

A large observer-blinded study comparing the utility of various techniques in the diagnosis of undifferentiated pediatric – round cell tumors[34,35] demonstrated that immunohistochemistry and electron microscopy when used in combination can be expected to produce a confident specific diagnosis in 96% of cases. The additional application of cytogenetic and molecular diagnostic techniques produced a diagnosis in another two percent. Thus it may be accepted that in rare instances, despite all efforts, a tumor will have to be categorized as "Unclassifiable"[55].

MATERIALS AND METHODS

The surgical specimens received in the department of Pathology, Institute of Child health, Egmore from the surgical department of the same institute during the period of 2007 Jan to 2008 December formed the material for this study. Small biopsy specimens and total excision specimens were included.

The clinical features such as age and sex of the patient, site of lesion and type of surgery done were noted.

The gross characteristics of the tumor included the tumor location, size, necrosis circumscription, cut section and secondary changes directly from the specimens.

Paraffin blocks were made and histological sections (5 to 6 micrometer) were routinely stained with hematoxylin and eosin stains. Microphotographs were taken. IHC stains were done wherever found necessary.

PROCEDURES

HEMATOXYLIN AND EOSIN

1. Dewax sections, hydrate through graded alchols to water.
2. Stain in Harris Hematoxylin for 5 minutes
3. Wash well in tap water
4. Diffentiate in 1% acid alcohol
5. Wash well in tab water until sections are again blue for 10-15 minutes
6. Stain in 1% eosin for 1 to 2 minutes
7. Wash in running tap water for 1 to 5 minutes
8. Dehydrate the alcohols clearly in Xylol and mount in DPX.

MICROSCOPIC FEATURES

The microscopic features were studied from all the available slides. These included the histological pattern, cellular features, pleomorphism, mitosis, vascularity and secondary changes. Diagnosis were made.

SPECIAL STAINS

Periodic Acid Schiff Stain (Glycogen in ES/PNET)

Method:

1. Dewax sections and bring to distilled water
2. Treat with periodic acid for 5 minutes
3. Wash well with several changes of dilateted water
4. Cover with Schiff's solution for 15 minutes
5. Wash in running tap water 5-10 minutes
6. Stain nuclei with Harris Hematoxylin. Differentiate as appropriate in acid alcohol and blueing in tapwater for 5 minutes.
7. Wash in water
8. Rinse in absolute alcohol
9. Clear in Xylene and mount with DPX

RESULTS

Glycogen : Magenta

Nuclei : Blue

IMMUNOHISTOCHEMISTRY

Cases for IHC were selected after viewing the H &E sections. Slides were coated with chrome alum, and subjected to Antigen Retrival using the Microwave technique with Citrate buffer solution .Slides were then treated by HRP (Horse radish peroxidase) polymer technique.

HRP POLYMER TECHNIQUE

The coated slides were taken through the following steps

1. Treatment with peroxidase block – for incubation of endogenous peroxidase in the tissue for 20 minutes, washed in PBS buffer for 5 mts.

2. Applications of power block O- to block non specific antigen – antibody reactions for 20 minutes. The excess power block was blot dried.

3. Applications of Primary antibody – Murine antibodies for 60 minutes. Washed in PBS buffer for 5 minutes.

4. Application of super enhancer for 30 minutes which increased the sensitivity of antigen -antibody reaction thereby enhancing the final reaction product.

5. Application of SS label – Secondary antibody from goat with the tagged horse radish peroxidase enzyme for 30 minutes. Washed in TRIS buffer.

6. Application of DAB (Diamino benzidine) Chromogen for 5 minutes – which was cleared by the enzyme to give the colored product at antigen sites. Washed in distilled water for 5 minutes.

7. The slides were then counter stained with hematoxylin .Slides were air dried and mounted with DPX (Dibutylphalate Xylene)

The following markers were done

CD45, CD3, CD20, CD99, Cytokeratin, S-100, Synaptophys in and Chromogranin.

OBSERVATION AND RESULTS

INCIDENCE

The total number of 194 neoplastic lesions were reported during the period of 2 years, 2007 –2008. The distribution of the tumors is given in the following Table – 9 (Fig.2).

TABLE - 9

System	No of Cases
Lymphoreticular	74
Gastro Intestinal Tract and Hepatobilliary	12
Endocrine	2
Renal	34
Soft Tissues	10
Bone	9
Germ cell tumors	25
RS	1
Sympathetic system	27
Total	**194**

Among these Small Round Cell Tumors constituted 72 cases.

FIGURE - 2

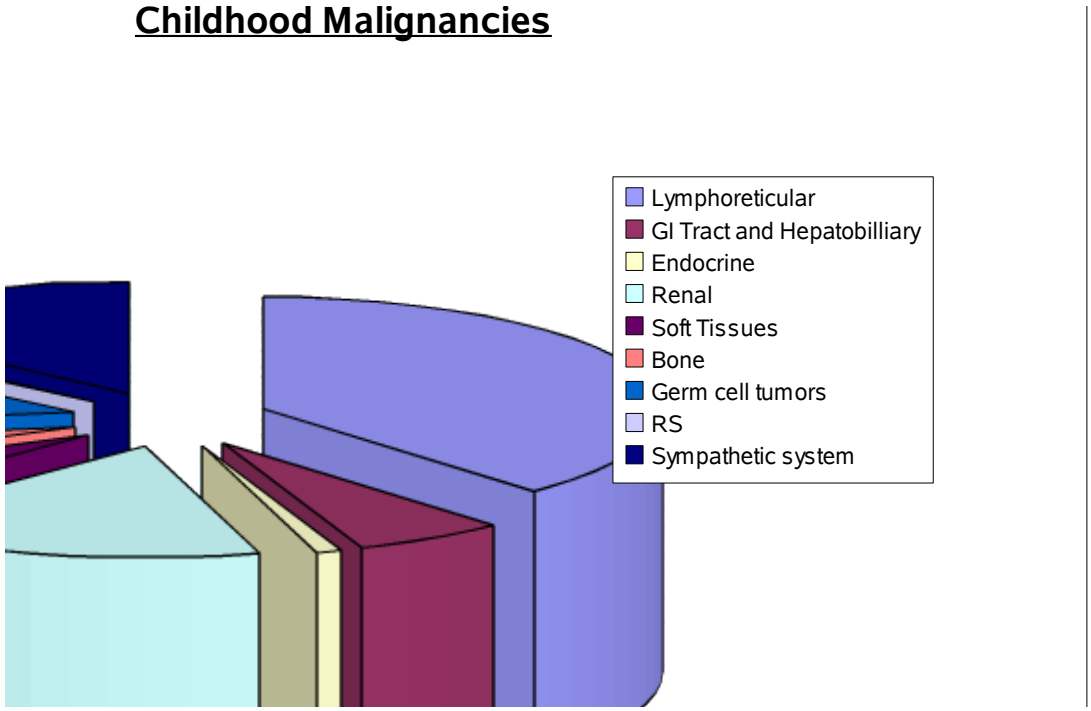
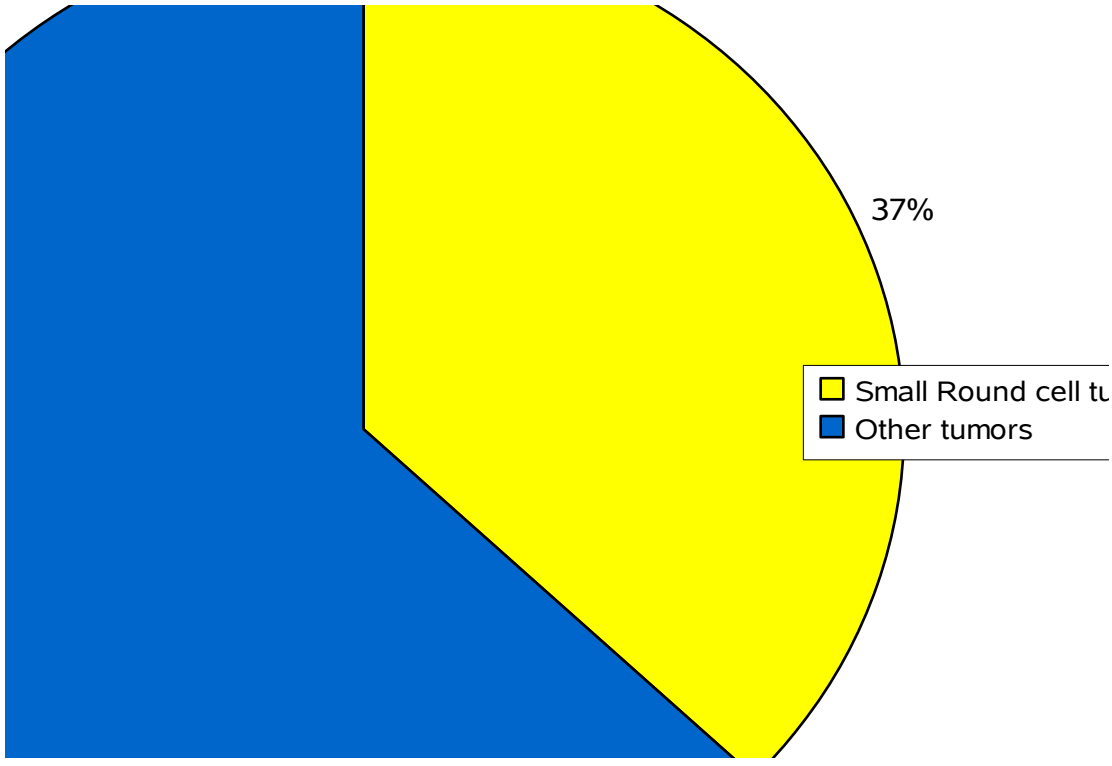


FIGURE - 3

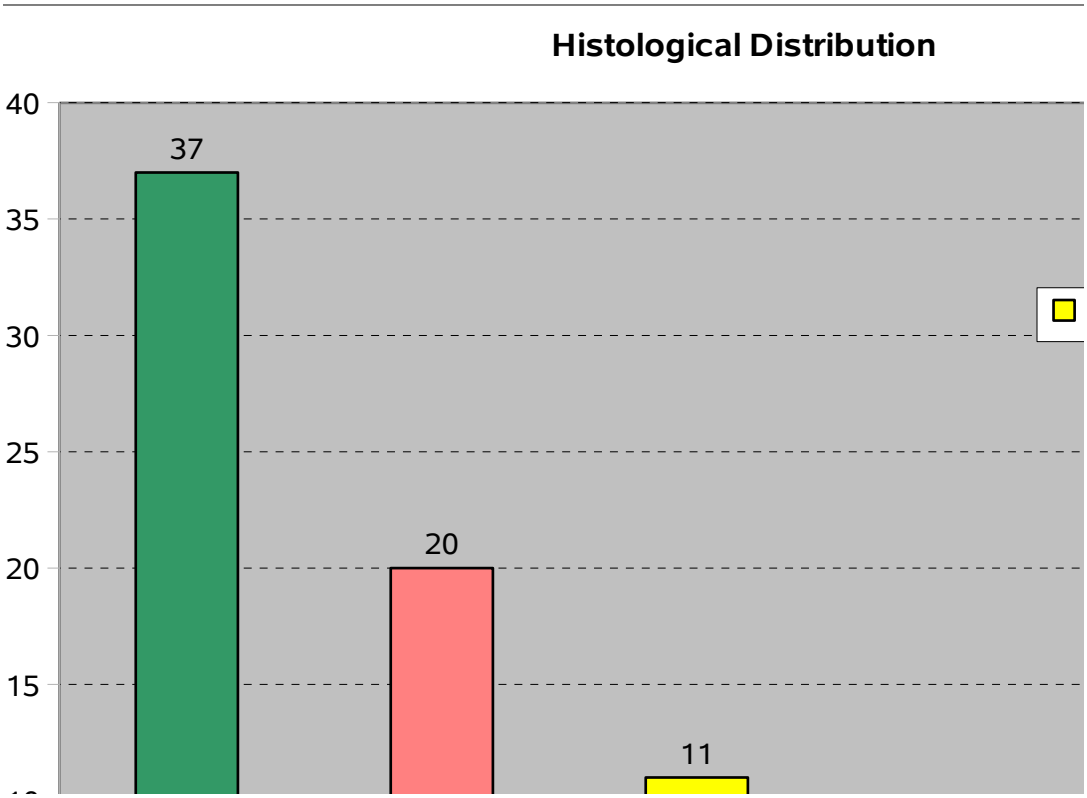


The Small Round Cell Tumors included the following Table – 10, (Fig.4):

TABLE - 10

Tumor Type	No of Cases	Percentage (%)
Non Hodgkins Lymphoma	37	51
Neuroblastoma	20	28
Ewings/PNET	11	15
Rhabdomyosarcoma	3	4
Wilms Blastemal Predominant	1	1
Total	72	100

FIGURE - 4



Non Hodgkins Lymphoma constituted almost 50% of the tumors followed by Neuroblastoma and Ewings sarcoma. Rhabdomyosarcoma constituted only 4% and Blastemal predominant Wilms constituted 1 %.

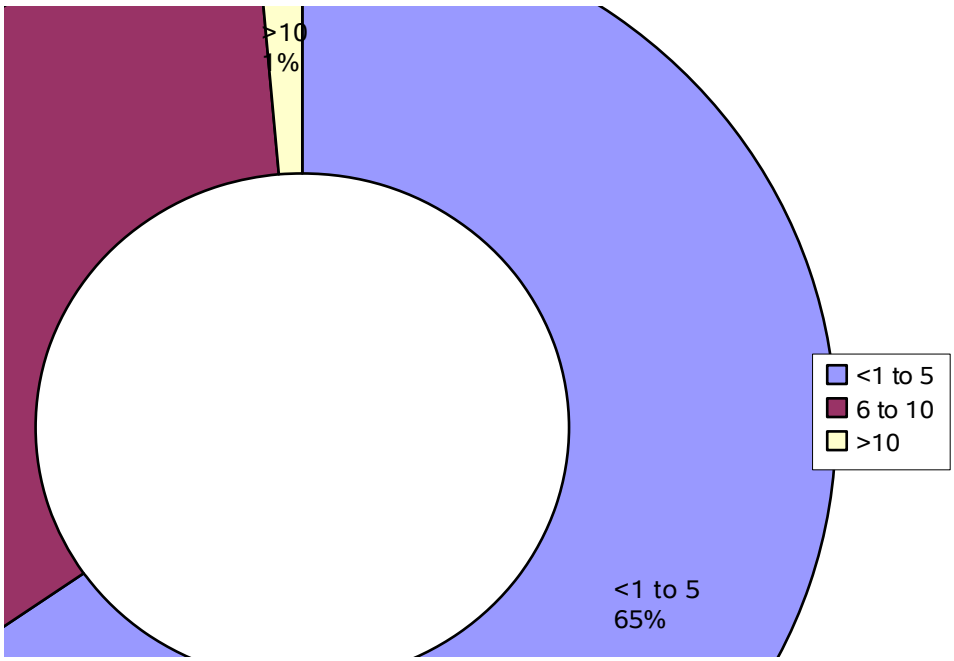
AGE DISTRIBUTION

Age of the patients with SRCTs ranged between < 1 Yr to 12 Yrs, in which 2/3rd of the cases were less than 5 Yrs (Table – 11, Fig.5).

TABLE - 11

Age Group	No. of Cases	Percentage (%)
<1 to 5	47	66
6 to 10	24	33
>10	1	1
Total	**72**	**100**

FIGURE - 5



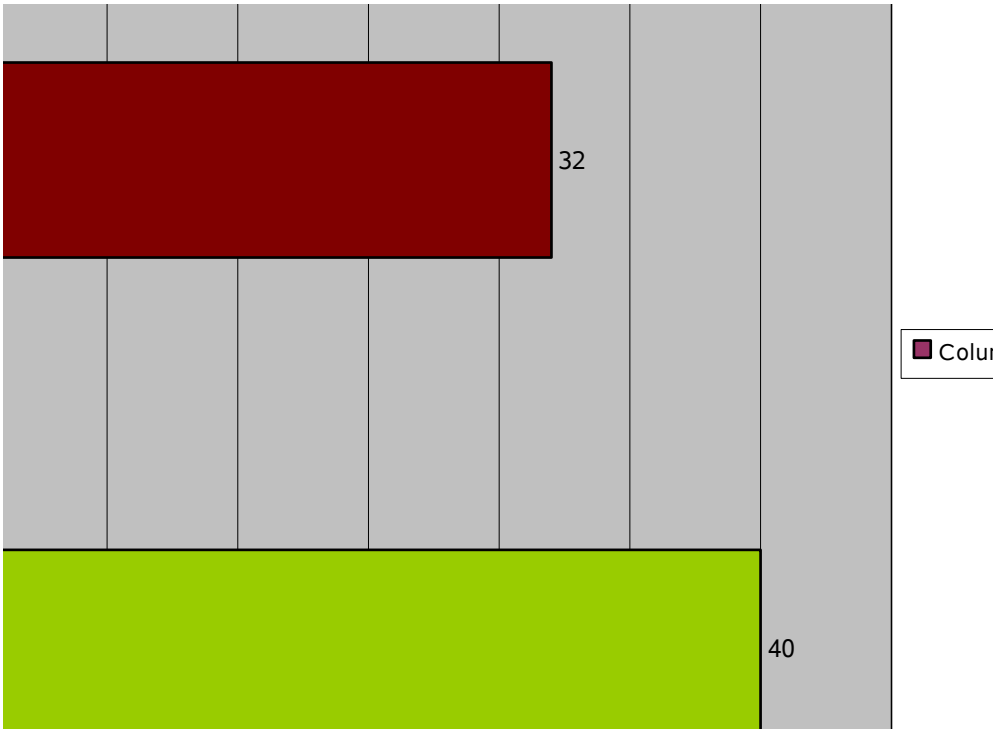
SEX RATIO

Among the 72 cases 40 were male patients and 32 were female. Thus there was a slight male predominance (Table 12).

TABLE - 12

Sex	No. of cases	Percentage
Male	40	55.6
Female	32	44.4
Total	**72**	**100**

FIGURE - 6



NON HODGKINS LYMPHOMA (NHL)

NHL constituted the most common tumor among the SRCTs with 37 cases out of the total 72 cases. Children of age group 2 to 5yrs were most commonly affected, the youngest being a one year old child (Table – 13). There was a male preponderance (Table – 14).

TABLE - 13

Age	**Cases**
1 to 5	22
6 to 10	14
> 10	1
Total	**37**

SEX RATIO

TABLE - 14

Male	**Female**
28	9

SYMPTOMS AND SIGNS

Children commonly presented with generalized lymphadenopathy. 5 of them presented with a mass abdomen.Fig.7 shows a tumor involving the small intestine. Fig.8, 9 and 10 show the microscopic features of marginal zone B cell lymphoma. 1 patient presented with a testicular mass, whose peripheral smear and bone marrow examination were normal. 1 child was a known case of ALL in remission who developed inguinal lymphadenopathy. On light microscopy a diagnosis of NHL was made. On IHC study it was found to be peripheral T cell lymphoma.

NEUROBLASTOMA

This was the second common SRCTs constituting around 28% of cases. Predominantly seen in age group of less than 5 yrs, among which 5 of them were less than 1yr (Table - 15. The youngest being a child presented with progressive distention of the abdomen from 10 days of birth. There was a female preponderance (Table – 16).

TABLE - 15

Age	Cases
1 to 5	17
6 to 10	3
> 10	0
Total	**20**

SEX RATIO

TABLE - 16

Male	Female
6	14

Among the 20 cases 13 of them were adrenal neuroblastomas. They usually presented as mass abdomen. Fig.21 shows adrenal Neuroblastoma infiltrating the adjacent renal parenchyma. One was a posterior mediastinal mass and two others presented as sacrococcygeal masses. Four cases were metastatic deposits, two of them in the lymphnodes, one in the liver and the other in the bone marrow. Grossly they had an average size of 5 x 5 cms with a bosselated external

appearance and the cut surface showed a homogenous whitish solid mass.

On light microscopy they showed small round cells arranged in true rosettes around central neuropil with schwanian stroma and calcifications. Most of the cases were poorly differentiated. Fig.22,23 and 24 show the microscopic features.

EWINGS/PNET

EWINGS sarcoma formed the third common sarcoma in the study, constituting 11 cases. There was a female preponderance, and the average age of children affected being 7 to 8 years (Table – 17 & 18). The youngest being a 3 month old child. More than 50% presented with chest wall swelling. Others with swelling in the extremities and paranasal sinuses.

TABLE - 17

Age	**Cases**
1 to 5	4
6 to 10	7
> 10	0
Total	**11**

SEX RATIO

TABLE - 18

Male	Female
4	7

On light microscopy the tumor cells are seen arranged in sheets, cords, filigree pattern. Fig.17 and 18 show the tumor cells arranged in nests separated by thin fibrous septae. The tumor exhibits dual population of neoplastic cells, small round cells with dark nuclei and scant cytoplasm and large polygonal cells with abundant clear cytoplasm and vesicular nucleus. Ill formed rosettes were also seen. Large fibrocollagenous and fibrillary stroma was seen in the background. PAS stain studied showed focal positivity.

RHABDOMYOSARCOMA

They constituted 3 cases among the 72 cases. All the children were less than 5 yrs old with a female preponderance (Table – 19 & 20). They presented with a swelling in extremities and head and neck.

TABLE - 19

Age	Cases
1 to 5	3
6 to 10	0
> 10	0
Total	**3**

SEX RATIO

TABLE - 20

Male	Female
1	2

On light microscopy 2 of them showed alveolar RMS and one was embryonal RMS. Fig.29 shows the alveolar pattern of tumor cells and Fig.30 shows rhabdomyoblast differentiation of the tumor cells.

BLASTEMAL PREDOMINANT WILMS TUMOR

Only one case diagnosed amongst 72 cases. It was a nephrectomy specimen from a 3 yr old boy. On light microscopy it showed a neoplasm arranged in nodules separated by spindle shaped stromal elements. The tumor cells were small round with dense nuclei. Few areas showed atrophic tubules. Fig.31, 32 and 33 show the microscopic features. Hence a diagnosis of Blastemal predominant wilms tumor was arrived at.

Immunohistochemistry was done randomly in 20 cases. Panel of CD99, LCA, CD20, CD3 and Cytokeratin were done to confirm EWINGS/PNET by histomorphology. Lymphoma panel comprising of CD45, CD20 and CD3 were done to confirm Non-Hodgkin's lymphoma.In 2 cases of Neuroblastoma a panel comprising of chromogranin, synaptophysin and S100 were done to confirm the diagnosis. Fig.5,6 and 9 show the positivity of CD45. Fig.7 and 10 show the membrane positivity of CD20.Fig. 13 and 14 show CD99 exhibiting membrane positivity.Fig.20,21 and 22 show the cytoplasmic positivity of S100, synaptophysin and chromogranin.

TUMORS IN WHICH IMMUNOHISTOCHEMISTRY HELPED IN CONFIRMING THE DIAGNOSIS

Case 1: 619/07, 10yrs old female child presented with mass abdomen with lymphnode enlargement in the right supraclavicular and suprascapular region. On histopathological examination of the lymphnode a neoplasm arranged in cords and nests separated by fibrocollagenous and fibrillary stromawas seen.A diagnosis of small round cell tumor was arrived at and IHC was suggested for confirmation. Following markers were done (Table – 21).

TABLE - 21

Markers	Results
CD99	Positive
Vimentin	Positive
MSA	Negative
Desmin	Focal Positive
Synaptophysin	Focal Positive
CD45	Negative

Consistent with Ewings sarcoma/PNET

Case 2: 806/07 3yr male child presented with axillary lymphadenopathy. X ray chest revealed mediastinal widening. Axillary node biopsy revealed diffused proliferation of lymphocytes, eosinophils and binuclear histiocytes. Hence the diagnosis of Hodgkins lymphoma was arrived at. Following markers were done (Table – 22).

TABLE - 22

Markers	**Results**
CD45	Focal Positive
LMP	Negative
CD5	Negative
CD20	**Focal Positive**
ACK-1	Positive
CD3	Negative
EMA	Positive
CD30	Positive

Consistent with Anaplastic large cell lymphoma

Case 3: 631/08 A 7yr female child presented with cervical lymphadenopathy. Lymphnode on histopathological examination showed features suggestive of chronic non specific lymphadenitis.However IHC was suggested to rule out lymphiproliferative disorder. Following markers were done (Table – 23).

TABLE - 23

Markers	**Results**
CD3	Positive
CD45	Positive
CD20	Negative
CD79a	Negative
Ki67	40 – 50%
Tdt	Positive
CD99	Positive

Consistent with T-cell lymphoblastic lymphoma/leukemia.

Case 4: 815/08 5yr male child presented with swelling in the right mandible region. X-ray mandible showed a lytic leision with soft tissue swelling. Histopathologic examination revealed a neoplasm arranged in sheets and attempted rosettes in a fibrillary background. Hence a diagnosis of small round cell tumors, probably Neuroblastoma/PNET was arrived at. Following markers were done (Table – 24).

TABLE - 24

Markers	**Results**
CD45	Positive
Vimentin	Focal Positive
CD56	Negative
Desmin	Negative
Synaptophysin	Negative
CD99	Negative
CD20	Positive
Ki-67	Increased, 56%
CD79a	Positive
Tdt	Negative

Consistent with High grade B lineage Non Hodgkins lymphoma

Case 5: 380/08 8yr old female child presented with retroperitoneal mass. X-ray revealed 12th rib sclerotic changes. Incisional biopsy was done. Histopathologic examination showed a neoplasm composed of cells arranged in lobules separated by fibrous septa. Impression was small round cell tumor suggestive of Ewings sarcoma was arrived at. Following markers were done (Table – 25).

TABLE - 25

Markers	**Results**
CD99	Positive
Vimentin	Positive
CD45	Negative
Cytokeratin	Negative

DISCUSSION

There has been enormous progress in the treatment of childhood cancer described. Hitherto, it is essential to systematically study the burden of childhood cancer in India and to understand how the occurrence and outcome of the disease varies across the country. A comprehensive review of medical literature revealed that 1.6 to 4.8% of all cancer in India is seen in children below 15 years of age and the overall incidence of 38 to 124 per million children, per year, is lower than that in the developed world. There is considerable inter-regional variation in incidence and mortality rates across India, this suggests a possible deficiency in ascertainment of cases and death notification, particularly in rural areas. The marked male preponderance of Hodgkin's disease, lower incidence of central nervous system tumors, and higher incidence of retinoblastoma merit further analysis.

In the Institute of Child Health the most common malignancy was Lymphomas, followed by germ cell tumors and Wilms tumor.

Pediatric small round cell tumors comprise a group of diverse, diagnostically challenging primitive or undifferentiated neoplasms.

Precise diagnosis of the tumor type is necessary for selection of appropriate treatment protocol.

Small Round Cell tumors constitute about 20% of the solid tumors in children. In this study the SRCTs constituted around 37%.This again because CNS tumors were not included,Taken collectively lymphomas are the third most common childhood malignancies after acute leukemias and brain tumors. It has a male predilection. This study substantiates tha fact.In this study NHL was the predominant tumor type constituting 37 cases. They formed 51% of the total cases. There was a male predominance with a M:F ratio 3.1:1 Children commonly presented with generalized lymphadenopathy. 5 of them presented with a mass abdomen. 1 patient presented with a testicular mass, whose peripheral smear and bone marrow examination were normal. 1 child was a known case of ALL in remission who developed inguinal lymphadenopathy. On light microscopy a diagnosis of NHL was made. On IHC study it was found to be peripheral T cell lymphoma.

Neuroblastoma is the third most common malignant tumor and the most common extracranial solid tumor, with a male predilection, generally diagnosed in early childhood, 50% are below 2yrs and 90% below 5yrs [x]. In this study also Neuroblastoma was the second common

small round cell tumor. Among the 72 cases, 20 cases were Neuroblastoma, thus constituting around 38.5% of all the SRCTs. Neuroblastoma involved children of age less than 5 years predominantly. 5 of them were less than one year. The youngest being a child who presented with progressive distention of abdomen from the 10th day of birth. However there was a female preponderance with 14 cases among the 20 cases.This was against the fact that Neuroblastomas have male predominance in studies by Enzinger et al and others.

Most of the cases were adrenal neuroblastomas presenting as mass abdomen. One was a posterior mediastinal mass. Two other presented with sacrococcygeal mass. 16 cases were primary neuroblastomas while 4 were secondary metastatic deposits to lymphnodes, liver and bone marrow.This goes well proved aspects of Neuroblastoma.

Ewings sarcoma formed the third common sarcoma in the study, constituting 11 cases. There was a female preponderance againt the male predominance in other studies and the average age of children affected being 7 to 8 years which goes well with oter studies. The youngest being a 3 month old child. More than 50% presented with chest wall swelling. Others with extremities and paranasal sinuses.

Rhabdomyosarcoma is the most common pediatric soft tissue sarcoma comprising 80-90% of reported sarcomas. Usually affects children before the age of 10yrs with a peak before the age of 4yrs. It has a male predilection (Fletcher). In our study it constituted 3 cases among the 72 cases. All the children were less than 5 yrs old with a female preponderance. They presented with a swelling in extremities and head and neck.

Wilms tumor with blastemal predominance has a histomorphology of small round cell tumor which leads to difficulty in diagnosis. In this study only one case was identified amongst 72 cases. It was a nephrectomy specimen from a 3 yr old boy. On light microscopy it showed a neoplasm arranged in nodules separated by spindle shaped stromal elements. The tumor cells were small round with dense nuclei. Few areas showed atrophic tubules. Hence a diagnosis of Blastemal predominant wilms tumor was arrived at.

Role of IHC

In many instances, the combination of the clinical presentation and the light microscopic appearance is sufficient to make a diagnosis. For example, a tumor in a baby with an adrenal mass and elevated catecholamine levels, which microscopically reveals neuroblasts, background neuropit, calcifications, and thin fibrovascular septa, can be comfortably diagnosed as a Neuroblastoma. However, an undifferentiated rhabdomyosarcoma may require immunohistochemistry or molecular

analysis to confirm the skeletal muscle differentiation of the tumor. The decision about when to order these special studies depends on the complexity of the tumor.

> In this study the most challenging was to differentiate betweenNon Hodgkins Lymphoma and Ewings Sarcoma.Neuroblastoma and Rhabdomyoblastomas had typical histomorphology.The help of IHC was saught to differentiate NHL and Ewings.

An initial limited panel is often the first step toward refining or confirming the diagnosis. If this results in unexpected findings, a second, more comprehensive panel may then be used. In both cases, however, a multiple antibody panel is usually used as reliance on one antibody can be misleading. For example, desmin-positive PNETs and CD99 positive synovial sarcomas are known to occur.

Interpretation of IHC findings requires proper understanding of the nature of expression of various markers in each tumors. Certain SRCTs express antigens based upon their degree of differentiation. For example with rhabdomyosarcoma, many different muscle precursor antibodies are available and may be needed for diagnosis due to the variable degree of myogenic differentiation in these tumors. Myogenic regulator gene proteins (myogenin, myoD1, mfy-3, myf-4) will be expressed as nuclear antigens at an earlier phase of muscle protein differentiation than those

associated with later cytoplasmic myogenic maturation (myoglobin). Myogenin, myoD1, poyclonal desmin, and muscle specific actin are expressed in over 90% of rhabdomyosarcomas; while, myoglobin is expressed variably (29 to 78%). In contrast, less than 5% of rhabdomyosarcomas immunoreact with smooth muscle actin.

Aberrant expression is not unusual in SRCTs. Rhabdomyosarcoma may be particularly troublesome, because CD99, CD19, CD20, cytokeratin and NSE may also be aberrantly expressed. This may lead to errant diagnoses of B-cell leukemia/lymphoma, Ewings sarcoma and Neuroblastoma in some cases of rhabdomyosarcoma that do not express typical myogenic marker. Tumors mimicking the light microscopic appearance of rhabdomyosarcoma, such as extra-renal rhabdoid tumor may require the addition of other markers (cytokeratin, EMA, vimentin in the case of rhabdoid tumor) to provide a definitive diagnosis. Lack of expression of tumor defining antigens may occur in SRCTs. For example in one subtype of rhabdomyosarcoma (undifferentiated sarcoma), vimentin may be the only tumor marker identified.

There is considerable cross-reactivity among various neoplasms and antibodies. For example, CD99 (MIC2) is not specific to Ewings sarcoma family of tumors alone. In fact, the following SRCTs immunoreact with CD99: lymphoblastic leukemia and lymphoma with a cytoplasmic pattern depending upon the lineage (60% unknown lineage, 75% B progenitor lineage, 100% T-cell lineage); desmoplastic small round cell tumor (DSRCT) with a membrane pattern. (DSRCT is characteristically polyphenotypic and immunoreacts with desmin in a "dot-like" or "globoid" pattern, neuron specific enolase and cytokeratin.) DSRCTs lacking desmin and cytokeratin staining may be mistaken for extraosseous Ewings sarcoma. Immunoreaction with WT-1 may be helpful in defining a DSRCT that does not express myogenic or epithelial markers and could be confused with Ewings sarcoma. A definitive diagnosis can be made on the basis of clinical, basic histologic and ancillary studies in virtually all cases of SRCTs. The critical aspects for accurate diagnosis of SRCT are adequate clinical data, adequate size of biopsy of tumor, expecting the unexpected if there are unusual clinical presentations (NHL arising in a bone or soft tissue, Burkitt's NHL presenting as small duodenal mucosal nodules) and apparent or real

discrepancies in the results of pathologic studies of biopsy and getting ancillary studies done.

Diagnosis of the precise type of SRCTs is rarely made on the basis of a single characteristic feature. Clinical and routine histopathologic features and results of ancillary studies are all taken into consideration.

SUMMARY AND CONCLUSION

During the period of study between January 2007 and December 2008 tumors with histomorphology of small round cells were taken up.194 neoplastic lesions were reported and among which SRCTS constituted 72 cases.

Among the SRCTs,Non Hodgkins Lymphoma was the most predominant type with 37 out of 72 cases, followed by Neuroblastoma with 20 cases, Ewings Sarcoma with 11 cases and Rhabdomyosarcoma with 3 cases.

Small Round Cell Tumors are commonly seen in children less than 10 years of age. The youngest was a 10 day old child diagnosed with Neuroblastoma followed by a 3 month old child with Ewings, 6 month old child with Rhabdomyosarcoma and 1 year old child with NHL. NHL had male predominance as in other studies. However Neuroblastoma, Ewings and Rhabdomyosarcomas had female predilection against the male predominance in previous studies.

Histomorphology was sufficient to diagnose most cases. Difficulties were faced in differentiating Ewings from NHL and to

differentiate NHL and HL.

Ancillary studies like IHC plays a key role in confirming a diagnosis. IHC markers CD45, CD20 And CD3 confirmed lymphomas, CD99 proved Ewings and Chromogranin, S-100 and Synaptophysin confirmed Neuroblastoma. However the limitations of IHC like aberrant expression of antigens and cross reactivity with other antigens should be kept in mind.

SRCTs are amongst the commonest solid tumors of childhood. They comprise a group of diverse, diagnostically challenging primitive or undifferentiated neoplasms,Precise diagnosis of the SRCTS depends on the clinical routine histopathologic features and results of ancillary studies like immunohistochemistry, electron microscopy, flow cytometry and molecular analysis.

BIBLIOGRAPHY

1. Denny C.Defining SRCTs of childhood. When is a rose, really a rose? J. Pediatric Hematology. Oncol 2001; 23; 338-339.

2. Joshi V.V. Small Cell Tumors and releated lesions in children. Chapter 12 in common problems in Pediatric Pathology. Joshi VV, Igaku Shoin Medical Publishers, Newyork, 1994 p 358-431

3. Parkin DM, Stiller CA, Draper GJ, Bieber CA. The international incidence of childhood cancer. Int J Cancer 1988;42:511-20.

4. Stiller CA, Parkin DM. Geographic and ethnic variations in the incidence of childhood cancer. Br Med Bull 1996;52:682-703.

5. Stiller C editor. Childhood cancer in Britain: Incidence survival, mortality. Oxford;Oxford University Press: 2007.

6. Gurney JG, Bondy ML. Epidemiology of childhood cancer. In: Pizzo PA, Poplack DG, Editors. Priciples and Practice of Pediatric Oncology, 5 th edition. Philadelphia; Lippincott Williams and Wilkins: 2006. P. 2-14.

7. Cancer in children (ages 0 – 14 and ages 0 –19). In Ries, LA, Kosary CL, Hankey BF, eds. SEER cancer statistics Review, 1973-74. NIH publication 97-2789, Bethesda, MD: cancer statistic Branch, surveillance Research Program, Division of cancer control and Population Sciences, National Cancer Institute, 1997.

8. Gurney JG, Davis S, Severson RK etal. Trends in cancer incidence among children in the US Cancer 1996; 78: 532

9. Srinivas V, Soman CS, Naresh KN. Study of the distribution of 289 non-Hodgkin lymphomas using the WHO classification among children and adolescents in India. Med Pediatr Oncol 2002;39:40-3.

10. Dinand V, Arya LS. Epidemiology of childhood Hodgkins disease: is it different in developing countries? Indian Pediatr 2006;43:141-7.

11. Swaminathan R, Rama R, Shanta V. Childhood cancers in Chennai, India, 1990-2001: incidence and survival. Int J Cancer 2008;122:2607-11.

12. Tyagi BB, Manoharan N, Raina V. Childhood Cancer Incidence in Delhi, 1996-2000. Indian J Med Paediatr Oncol 2006;27:13-8.

13. Arora RS, Eden T, Kapoor G. Epidemiology of childhood cancer in India. Indian J Cancer [serial online] 2009 [cited 2009 Nov 8];46:264-73.

14. Cancers in Childhood, Consolidated report of the HBCRs: 2001-2003.

15. Esiashvili N, Goodman, M.Ward K et al. Neuroblastoma in adults: incidence and survival analysis based on SEER data. Pediatric Blood cancer 2006.

16. Enzinger, soft tissue Tumors, 5th edition chapter 31, page 947.

17. Silverberg; Principles and Practice of Surgical Pathology and Cytopathology, 4th edition.

18. Dehner Pediatric Pathology, Second Edition.

19. Fletcher Diagnostic Histopathology of Tumors, Third edition

20. Kinney Mc, Kadin ME: The pathologic and clinical spectrum of anaplastic large cell lymphoma and correlation with ALK gen dysregulation. Am J clin Pathol 1999; 111 (Suppl): S56 – 67

21. Lombart – Bosch A, Contesso G, Peydio-olaya A: Histology, Immunohistochemistry and electron microscopy of small round cell tumors of bone. Semi DIAGNOSTIC Pathol 1996; 13: 153-70

22. d'Amore ESG, NinfoVF: soft issue small round cell tumors: morphological parameters. Sem Diasgnostic Pathol 1996; 13: 184-203

23. Coffin CM, Dehner LP: Pathologic evaluation of pediatric soft tissue tumors. Am J Cun Pathol 1998; 109 (Suppl 1): S38-52

24. Pinto A, Tallini G, Novak RW, Bowen T, Parham DM: Undiffentiated rhahdomyosarcoma with lymphoid phenotype expression. Med pediatr Oncol 1997; 28: 165-70

25. Wang NP, marx J, McNutt MA, Rutledge JC, Gown AM: Expression of myogenic regulatory proteins (myogenin and myoDI) in small blue cell tumors of childhood. Am J Pathol 1995; 147: 1799-810.

26. Riopel M, Dickman PS, Link mp, Perlman EJ: MIC2 analysis in pediatric lymphomas and lukemias: Hum Pathol 1994; 25: 396-9

27. Perlman EJ, Dickman PS, Askin FB, Grier HE, Miser JS, Link mp: Ewings sarcoma – routine diagnostic utilization of MIC2 analysis: a pediatric oncology group/children's cancer group intergroup study. Human Pathol 1994; 25: 304-7

28. Folpe AL, Schmidt RA, Chapman D, Gown AM; Poorly differentiated synovial sarcoma. Immunohistochemical distinction from primitive neuroectodermal tumors and high grade malignant peripheral nerve sheath tumors. Am J Surg Pathol 1998; 22: 673-82

29. Hibshoosh H, Lattes R: Immunohistochemical and molecular genetic approaches to soft tissue tumor diagnosis: a primer. Sem Oncol 1997; 24: 515-25

30. Ordonez NG: Desmpplastic small round cell tumors II: an ultrastructural and immunohistochemical markers. Am J Surg Pathol 1998; 22:1314-27

31. Quantman SJ, Coffin cm, Newton WA, Hojo H, Triche TJ, Parham Om, Crist WM: Intergroup rhahdomyosarcoma study: update for pathologists. Pediatr Develop Pathol 1998;1:550-61

32. Liapis H, Marley EF, Liny, Dehner Lp: p53 and ki-67 proliferhing cell nuclear antigen in benigh and malignant peripheral nerve sheath tumors in children. Pediatirc Develop Pathol 1999;2S: 377-84

33. Pappo As, Parham DM, Rao BN, Lobe TE; soft tissue sarcomas in children. Sem Surg Oncol 1999; 16:121-43

34. Malonem: Soft tissue tumors in childhood. Histopathol 1999; 23:203-16

35. M.E Woo S., Malawer, M.M and Hicks, M.J:Ewings sarcoma Family of Tumors: Ewings sarcoma of Bone and Soft tissue and the Peripheral Primitive Neuroectodermal Tumors. In principals and Practice of Pediatric Oncology, Third Edition. P.A.Pizzo and D.G. Poplack, Editors. Lippincott, Medical Phublishing, Philadephia, PA Chapter 31 pp 831-63, 1997

36. Hicks J, Quinones J.Rudman D, Smith B, Hawking E.Tumor suppressor gene protein and proliferation marker expression in Wilms Tumor Lab Invest 1999; 79: 179a(#1050)

37. Linden MD, Torres FX, Kubus J, Zarbo RJ: clinical application of morphologic and immunocytochemical assessements of cell proliferation. AM J. Cun Pathol 1992; 97 (Suppl) S4-13

38. Bozzola JJ, Russell LD, Electron Microscopy. Principles and Techiguws for Biologists. Sudhury, Massadhusetts: Jones and Bartlett Publishers, 1992

39. Johannessen JV. Use of paraffin material for electron microscopy. Pathol Ann 1977; 12: 189-224

40. Strausbauch P, Neill J, Dabbs DJ, Silverman JF. The impact of fine needle aspiration biopsy on a diagnostic electon microscopy laboratory. Arch Pathol Lab Med 1989; 113: 1354-1356

41. Yazdi HM, Dardick I, Diagnostic Immunocytochemistry and Electron Microscopy. Newyork, NY; Lgaku-shoin, 1992 (Kline Ts, ed.Guides to clinical Aspiration Biopsy).

42. Mierau GW, Berry PJ, Orsini EW, Jr.Small Round Cell neoplasms: Can Electon Microscopy and immunohistochemical studies accurately classify them? Ultrast.Pathol 1955;9:99-111

43. Mierau GW. Electon Microscopy in tumor diagnosis: is it redundant? Histopathology 1999;35: 99-101

44. Mierau GW, Berry PJ, Malott RL, Weeks DA. Appraisal of the comparative utility 06 immunohistochemistry and electron microscopy in the diagnosis of childhood round cell tumors. Ultrastruct Pathol 1996; 20: 507-517

45. Mierau GW, Weeks DA, Hicks MJ. Role of electron microscopy and other special techniques in the diagnosis of childhood round cell tumors. Human Pathol 1998; 29:1347-1355.

46. Tsongalis GJ, Coleman WB. Molecular oncology: Diagnostic and prognostic assessment of human cancers in the clinical laboratory. Cancer Invest 1998; 16:485-502

47. Brodeur GM. Molecular pathology of human neuroblastomas seminars Diagn Pathol 1994; 11:118-125

48. Sorensen PHB, LiuXF, Delattre O, Rowland JM, BiggsCA, Thomas, Triche TJ, Reverse transcriptase PCR amplification of EWS/FLI-1 fusion transcripts as a diagnostic test for peripheral primitive neuroectodermal tumors of childhood. Diagn Mol Pathol 1993; 2: 147-157

49. Zoubek A, Pflerderer C, Salzer-Kuntschik M, Amann G, Windhager R, Fink FM, Koscrelneak E, Delattreo, Strehis, Ambros PF, Gadner H, Kovar H. variablility of EWS chimaeric transcripts in Ewngs tumors: a comparison of clinical and moledular data. Br J cancer 1994; 79: 908-913

50. Rezuke WN, Abernathy EC, Tsongalis GJ. Molecular diagnosis of B and T cell lymphomas: fundamental principles and clinical applications. Clin chem. 1997; 43: 1814-1823

51. Birch JM, Brestow N. Epidemiologic features of Witm's tumor. Menatol oncol linics North Americal 1995;9:1157-1178

52. Biegel JA, Conrad K, Brooks JJ. Translocation (11:22) (p13:q12) primary change in intra-abdominal desmoplastic small round cell tumor. Genes chromosome cancer 1993;7:119-121

53. Barr FG, Nauta LE, Hollows Jc. Structural analysis of PAX & genomic rearrangements in alveolar rhahdomyosarcoma. Cancer Gene to cutogent 1998;102:32-39

54. Van de Rijn M, Barr FG, Collins MH, Xiong QB, Fisher C.Absence of SYT-SSX fusion products in soft tissue tumors other than synovial sarcoma. AM J cun Pathol 1999; 112: 43-49

55. Dehner LP. On Trial: A malignant small round cell tumor in a child. Four wrongs don not make a right. Am J Cun Pathol 1998;109:662-668

MASTER CHART

S. No.	HPE No.	Age	Sex	Site	HPE Diagnosis
1	1/07	6	M	Cervical LN	NHL-High grade
2	15/07	3	M	Mass abdomen	NHL-High grade
3	21/07	8/12	M	L forearm mass	Ewings sarcoma
4	24/07	8	F	L forearm mass	Ewings/PNET
5	141/07	4	F	Mass abdomen, Mesenteric LN	NHL-High grade
6	170/07	1 1/2	M	Mass abdomen	Neuroblastoma differentiating
7	193/07	5	M	Axillary & Inguinal nodes	NHL-High grade
8	203/07	12	F	Mass abdomen	NHL-Diffuse large cells
9	222/07	2 1/2	F	R chest mass	PNET
10	243/07	4	F	Bone marrow	Neuroblastoma-secondary deposits bone marrow
11	254/07	3 1/4	F	Mass abdomen	NHL-High grade
12	269/07	3	F	L maxillary swelling	ERMS
13	279/07	4	M	R chest wall LN	NHL-High grade
14	416/07	5	F	Mass abdomen	NHL-High grade
15	458/07	6	F	Generalized LN	NHL-Diffuse large cell
16	464/07	7	M	Cervical LN	NHL-High grade
17	465/07	1	F	Generalized LN	NHL-Diffuse large B cell
18	545/07	7	M	Generalized LN	NHL-High grade
19	559/07	6	M	L adrenal tumor	Neuroblastoma differentiating
20	601/07	9	F	Mass abdomen	Neuroblastoma differentiating
21	606/07	7	M	Axillary nodes	NHL-Diffuse larger B cell
22	619/07	10	F	R chest wall mass	PNET
23	631/07	5	M	Testicular mass	NHL-Diffuse large B cell
24	729/07	33/365	M	Liver biopsy, mass abdomen	Neuroblastoma
25	806/07	3	M	L axillary node bx, Generalized LN	Anaplastic Large cell Lymphoma
26	880/07	7	M	Generalized LN	NHL High Grade
27	922/07	4 1/2	F	R adrenal mass	Adrenal Neuroblastoma undifferentiated
28	926/07	5	M	Cervical LN	NHL-High grade Burkitts

S. No.	HPE No.	Age	Sex	Site	HPE Diagnosis
29	1034/07	1 1/4	M	Mass abdomen	Neuroblastoma
30	1052/07	2 1/2	M	Sacrococcygeal mass	Neuroblastoma undifferentiated
31	1086/07	9	F	L chest wall mass	PNET
32	1170/07	5	F	Paraaortic nodes	NHL- Diffuse large B cell
33	1321/07	4 1/2	M	Mass abdomen	NHL-High grade
34	1349/07	2 1/2	M	Adrenal mass	Neuroblastoma poorly differentiated
35	1368/07	11	F	R clavicle	Ewings sarcoma
36	1393/07	10	M	L inguinal nodes	NHL-High grade
37	1422/07	1 1/2	F	Ladrenal mass	Neuroblastoma poorly differentiated
38	1427/07	7	F	Generalized LN	NHL-Diffuse large cell
39	16/08	10	M	Generalized LN	NHL-Diffuse large B cell
40	23/08	4	M	R calf muscle posterior	Alveolar RMS
41	73/08	4 1/2	F	Mass abdomen	Neuroblastoma poorly differentiated
42	78/08	4	M	Mass small bowel	NHL High grade
43	85/08	10	M	Generalized LN	NHL-High grade
44	88/08	1	M	L lumbar mass	Neuroblastoma undifferentiated
45	100/08	4	M	R cervical LN	NHL-High grade
46	227/08	1	F	Presacral mass	Neuroblastoma poorly differentiated
47	287/08	1	F	Pelvic mass	Neuroblastoma differentiated
48	380/08	8	F	Retroperitoneal mass	Ewings sarcoma
49	396/08	3	M	Generalized LN	NHL
50	458/08	7	M	Cervical LN	NHL-High grade
51	480/08	7	M	Chest mass	Ewings / PNET
52	517/08	5	M	Generalized LN	NHL-High grade
53	566/08	7	M	Generalized LN	NHL-High grade
54	579/08	1 2/12	F	Mediastinal node	Neuroblastoma deposit lymphnode
55	582/08	8/12	F	Pelvic mass	Alveolar RMS
56	591/08	9	F	L axillary node	Neuroblastoma deposit lymphnode
57	631/08	7	F	Generalized LN	T cell lymphoblastic lymphoma/Leukemia
58	663/08	8	M	Small intestinal mass	Marginal zone B cell lymphoma
59	786/08	5	M	Generalized LN	NHL-Lymphoblastic

S. No.	HPE No.	Age	Sex	Site	HPE Diagnosis
					lymphoma
60	808/08	3 1/2	M	L side of face	PNET
61	815/08	5	M	R mandible mass	NHL
62	921/08	3	F	Nephrectomy	Neuroblastoma-Differentiating
63	942/08	8	M	Generalized LN	NHL
64	960/08	7	M	R femur	Ewings/PNET
65	975/08	4 1/2	M	Generalized LN	NHL- High grade
66	1024/08	3	M	Nephrectomy	Wilm`s tumor- blastemal predominance
67	1074/08	1 1/2	F	Posterior mediastinal mass	Neuroblastoma differentiating type
68	1137/08	3	F	Abdominal mass	Neuroblastoma differentiating type
69	1257/08	3	M	Generalized LN	NHL High grade
70	1381/08	4	F	Retropancreatic mass	Neuroblastoma poorly differentiated
71	1431/08	5	M	Generalized LN	NHL High grade
72	1432/08	4	M	Nasal cavity mass	PNET

ANNEXURE - 1

IHC RESULTS

NHL High Grade

SNo	HPE No	IHC Markers	Results	Diagnosis
1	15/07	LCA CD20 CD3	Positive Positive Negative	***Non Hodgkins Lymphoma B lineage***
2	806/07	LCA CD5 CD20 LMP ACK EMA CD3 CD30	Focal Positive Negative Positive/Negative Negative Positve Positive Negative Positive	***Anaplastic Large Cell Lymphoma***
3	1393/07	CD3 CD20 CD79a CD43 TdT CD99 Ki67 CD30	Positive Negative Negative Positive Negative Positive 50 to 60% Negative	***Peripheral T cell Lymphoma***
4	16/08	CD45 CD20 CD3	Positive Positive Negative	***Non Hodgkins Lymphoma B lineage***
5	458/08	CD45 CD20 CD3	Positive Positive Negative	***Non Hodgkins Lymphoma B lineage***
6	631/08	CD3 CD43 CD20 Ki67 TdT CD99	Positive Positive Negative 40 to 60% Negative Positive	***Peripheral T cell Lymphoma***
7	815/08	LCA Vimentin Synaptophysin CD56 Desmin CD99 Ki67 CD20 CD79a TdT	Positive Positive Negative Negative Negative Negative About 56% Positive Negative Negative	***Non Hodgkins Lymphoma High Grade B Lineage***
8	885/08	CD45 CD20 CD3	Positive Positive Negative	*Non Hodgkins Lymphoma B lineage*
9	1427/08	CD45 CD20 CD3	Positive Positive Negative	***Non Hodgkins Lymphoma B lineage***
10	663/08	CD45 CD20 CD3	Positive Positive Negative	***Non Hodgkins Lymphoma B lineage***

Ewings Sarcoma

SNo	HPE No	IHC Markers	Scoring	Diagnosis
1	480/08	CD99 CD45 Cytokeratin Vimentin	Positive Negative Negative Positive	**Ewings/PNET**
2	21/07	CD99 CD45 Cytokeratin Vimentin	Positive Negative Negative Positive	***Ewings/PNET***
3	619/07	CD99 Vimentin MSA Desmin Synaptophysin	Positive Positive Negative Positive/Desmin Positive/Negative	***Ewings/PNET***
4	380/08	CD99 CD45 CD20 CD3 Cytokeratin	Positive Negative Negative Negative Negative	***Ewings/PNET***
5	960/08	CD99 LCA Vimentin Cytokeratin Desmin Synaptophysin CD58	Positive Positive Negative Positive Negative Negative Negative	***Ewings/PNET***

Neuroblastoma

Sno	HPE No		IHC Markers	Results	Diagnosis
1	921/08		Chromogranin Synaptophysin S100	Positive Focal Positive Positive	**Neuroblastoma**
2	1381/08		Chromogranin Synaptophysin S100	Positive Positive Positive	***Neuroblastoma***